Summary

The malaria vector landscape across Africa is undergoing rapid change and entomologists in the LSHTM Malaria Centre are rising to meet the challenge. In East Africa, *Anopheles arabiensis* has become more prevalent relative to its sibling species *Anopheles gambiae.* Along coastal Tanzania *Anopheles gambiae* has declined in density, and this coincides with a fall in clinical malaria recorded in local hospitals. Meanwhile there is growth of pyrethroid resistance in *Anopheles gambiae* which is no longer restricted to West Africa but is detectable in several East African countries and growing in frequency with each passing year. An important driver of change – besides environmental factors - is the scaling up of vector control and the increasing coverage of Long Lasting Insecticide-treated Nets (LLINs) and Indoor Residual Spraying (IRS). These interventions are having a major impact on malaria burden but perhaps inevitably are also affecting the dynamics and evolution of mosquito vectors. There is tantalising evidence that *Anopheles arabiensis* is less readily killed by an LLIN than *Anopheles gambiae*, owing to behavioural differences between the species, and is perhaps one reason why *Anopheles arabiensis* seems more evident while *Anopheles gambiae* declines.

In Benin in West Africa, it is shown that in pockets of high pyrethroid resistance that Insecticide Treated Nets (ITNs) provide less protection to families as demonstrated by reduced mosquito mortality rates and higher blood feeding rates higher compared to areas with little or no resistance. Is this a problem now occurring in East Africa? In Northwest Tanzania there is a surprisingly high prevalence of malaria in some areas despite several rounds of IRS.

There is interest in the potential of combination interventions to reduce transmission over and above what IRS or LLIN can achieve individually. Community randomised trials of LLIN and IRS with carbamate insecticides are being sponsored by USAID to control pyrethroid resistant populations in Northwest Tanzania. The Innovative Vector Control Consortium, supported by The Bill & Melinda Gates Foundation, has funded small scale funding trials of LLIN plus IRS with novel insecticides such as chlorfenapyr and these combination interventions seem to restore vector control where previously pyrethroid resistance was undermining recent gains. The race is on to identify alternative insecticides to pyrethroids and to make these long lasting through reformulation. Two organophosphate insecticides have been given a new lease of life through reformulation, and one of these, *pirimiphos methyl*, offers the prospect of several months of vector control with a single application. Aside from improving formulations, members of the Malaria Centre are also investigating alternative substrates for applying insecticide in the

home. Durable wall lining, a form of polyethyelene sheeting that was first developed for use as refugee shelter in displacement camps, can be impregnated with insecticide during manufacture and used to line the interior walls of habitations to serve as both decoration and long lasting IRS. Impregnated with pyrethroid, durable wall lining works like any pyrethroid IRS, but for longer periods. But treated with a non-pyrethroid insecticide it offers the prospect of controlling pyrethoid resistant mosquitoes. Another simple but promising alternative substrate for applying chemical control agents are wall hangings made from curtains or netting material. Appropriate technology is not limited to chemical control. Work with spatial repellents, once again sponsored by The Bill & Melinda Gates Foundation, may circumvent the need for discipline in applying topical repellent and drive mosquitoes away from domestic interiors. Some repellents come from natural sources. Lantana camara is a mosquito repelling plant which can be arranged around houses to reduce house entry by vector mosquitoes by 56-83% depending on the species. Repellent treated sheets and blankets to provide additional control over that achievable with LLINs are being investigated in cluster randomised trials.

While the malaria vector landscape continues to evolve and produce new challenges, members of the Malaria Centre are working to address these, though a mixture of ingenuity and pragmatism, working with southern partners through field sites in Tanzania, Kenya, Benin and the Gambia and though alliances such as the Malaria Transmission Consortium, Pan African Malaria Vector Research Consortium, the President's Malaria Initiative, WHO Pesticide Evaluation Scheme and Medical Research Council.

Mosquito net.

Malaria Transmission Consortium (MTC) in the Western Kenyan Highlands.

***LSHTM Investigators**: Jonathan Cox, Chris Drakeley, Jennifer Stevenson, Mary Cooke*

***External Investigators/Collaborators:** Mary Hamel, John Gimnig, Kayla Laserson, John Vulule & Nabie Bayoh (KEMRI/CDC, Kenya); Neil Lobo, Brandy St. Laurent, Frank Collins (University of Notre Dame, USA); Ralph Harbech (Natural History Museum, UK).*

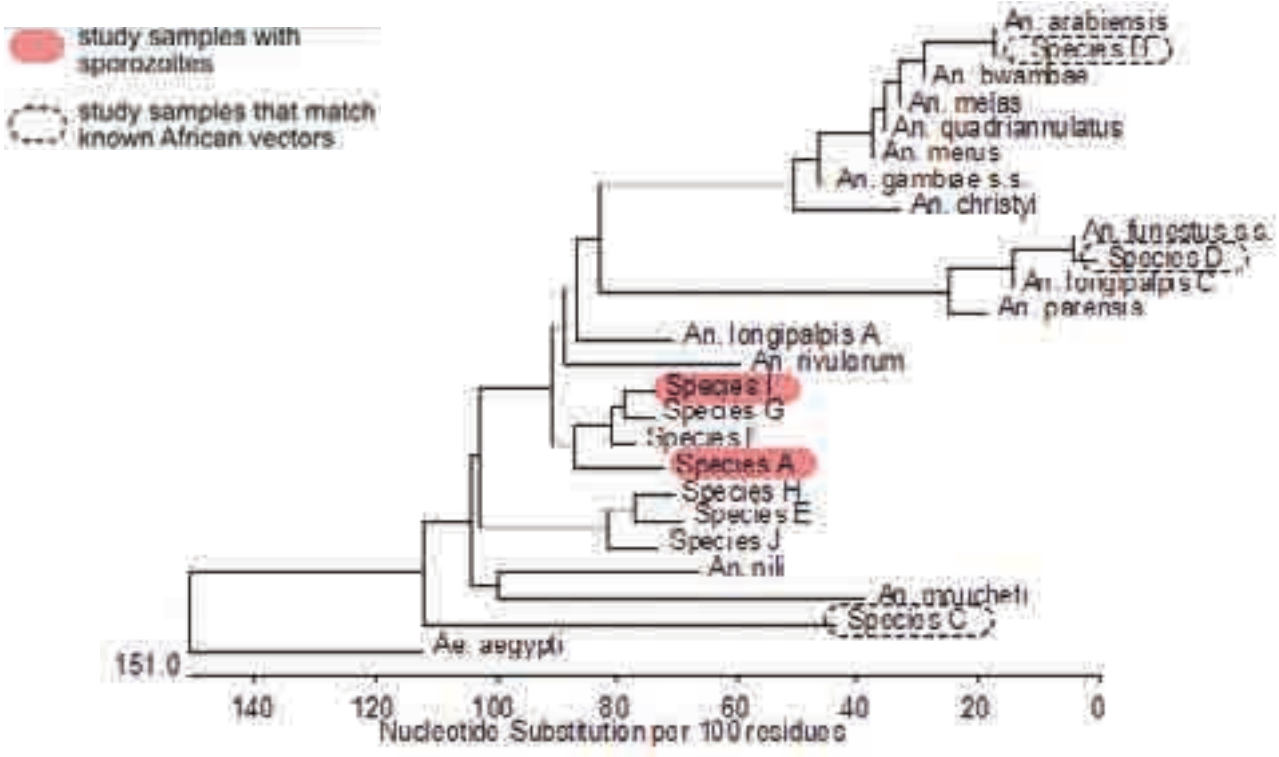
***Funding Body:** The Bill & Melinda Gates Foundation through the Malaria Transmission Consortium.*

MTC activities in Western Kenya encompass a range of sub-projects that together address the need to develop standardized measures of malaria transmission as a means of assessing the effectiveness of programmatic interventions in unstable transmission settings. This has included studies comparing a range of evaluation approaches (community and school cross-sectional surveys, community cohort studies, health facility monitoring and entomological monitoring).

As part of this work a Latin-square design experiment was carried out in 2010 to compare the suitability of different vector trapping methods in a highland fringe setting. In the event a large proportion of the mosquitoes caught could not be identified definitively using available morphological keys or by using diagnostic PCR for *Anopheles gambiae.* Subsequent genetic sequencing of ribosomal ITS2 and mitochondrial CO1 regions revealed that the majority of the mosquitoes, including those with malaria sporozoites, had unique unpublished sequences dissimilar to all known malaria vectors in the area. Significantly, the most abundant clade of unidentified mosquitoes (42% of the specimens sampled) was trapped outdoors and before 22:30 hours. We are continuing to work with our collaborators at Notre Dame University to sequence mosquitoes caught by pyrethrum spray catch from across the district.

In late 2011 collaborators from the Natural History Museum collected and reared over 500 *Anopheles* larvae through to adult stages from the study sites. Sequencing of the ITS2 and CO1 region by Notre Dame collaborators will be linked to full morphological descriptions of the samples to fully describe any unusual species from the area.

A longitudinal survey is now ongoing to determine the relative importance of specific species (including newly-identified species) in terms of malaria transmission and to investigate species-specific host-seeking behaviours. Initial data support observations that a substantial proportion of host seeking mosquitoes are sampled outdoors in the evening . This has potentially important implications in an area where vector control (in the form of IRS and LLINs) is predicated on the expectation of indoor, night-time biting.



Phylogenetic tree of sequence group consensuses with NCBI reference sequences. Samples caught arbitrarily named species 'A' to 'I', ranked by abundance.

Summary table of genetic and morphological identification of female Anopheles caught in Kisii, Kenya.

Sequence Group	ITS2 sequence homology	COI sequence homology	Closest species from morphological key (4)	No. female (% total catch)	No. tested for *P.falciparum* sporozoites	No. with sporozoite s (% sequence group)
Species A	-	-	*An. funestus/ An. demeilloni*	147 (42.2)	129	4 (3.1%)
Species B	*An. arabiensis*	*An. arabiensis*	*An. gambiae*	74 (21.3)	61	0
Species C	-	*An. coustani*	*An. coustani*	33 (9.5)	28	0
Species D	*An. funestus*	*An. funestus*	*An. funestus*	25 (7.2)	22	0
Species E	-	-	*An. maculipalpis*	22 (6.3)	18	0
Species F	-	-	Mixed	12 (3.4)	8	0
Species G	-	-	Mixed	13 (3.7)	9	0
Species H	-	-	Mixed	9 (2.6)	6	0
Species I	-	-	*An. harperi*	8 (2.3)	7	1 (14.3%)
Species J	-	-	Mixed	5 (1.4)	4	0
			Totals	348	292	5 (1.7%)

Pan African Malaria Vector Research Consortium (PAMVERC).

***LSHTM Investigators**: Richard Oxborough & Mark Rowland.*

***External Investigators/Collaborators:** Franklin Mosha (Kilimanjaro Christian Medical University College, Tanzania).*

***Funding Body:** The Bill & Melinda Gates Foundation through the Innovative Vector Control Consortium.*

The London School of Hygiene and Tropical Medicine in partnership with Kilimanjaro Christian Medical University College conducted a series of insecticide evaluations of new products for malaria vector control.

Pyrethroids are the only group of insecticides approved by World Health Organization for treating mosquito nets (ITN) and have also been preferred for Indoor Residual Spraying (IRS) in Africa in recent years. Consequently, pyrethroid resistance has become widespread in malaria vectors across Africa. For continued effectiveness of malaria control programmes it is imperative to develop new formulations of insecticides for ITN and IRS.

A series of trials were done in collaboration with several major chemical manufacturers including BASF, Bayer, DuPont, Sumitomo and Syngenta. Trials have focused on Phase 1 laboratory and Phase 2 experimental hut evaluation of novel products for the control of pyrethroid resistant mosquito populations.

Several novel long-lasting or wash resistant formulations have produced promising results and data has been submitted to the World Health Organization Pesticide Evaluation Scheme as part of product registration.

There is still a major dearth of public health insecticides and in future we will continue evaluating promising new products at all stages from laboratory to community testing, as well as investigating resistance management strategies.

We expect that some of the products we have evaluated will be used for widespread malaria vector control in Africa within the next few years.

Species shifts in the Anopheles gambiae complex: do LLINs successfully control Anopheles arabiensis?

***LSHTM Investigators**: Richard Oxborough, Jane Bruce & Mark Rowland.*
***External Investigators/Collaborators:** Franklin Mosha & Jovin Kitua (Kilimanjaro Christian Medical University College, Tanzania); Stephen Magesa, Robert Malima & Patrick Tungu (National Institute for Medical Research, Tanzania).*

***Funding Body:** The Bill & Melinda Gates Foundation through the Gates Malaria Partnership.*

Coverage of conventional and Long-Lasting Insecticide treated Nets (ITNs and LLINs) in parts of East Africa are associated with reductions in local malaria burdens.

Between 2005-2006 six experimental hut trials of ITNs and LLINs were conducted in parallel at two field stations in northeastern Tanzania; the first was in Lower Moshi Rice Irrigation Zone, an area where *Anopholes arabiensis* predominates, and the second was in coastal Muheza where *Anopholes gambiae* and *Anopholes funestus* predominate. Five pyrethroid and one carbamate insecticide were evaluated on nets in terms of insecticide-induced mortality, blood-feeding inhibition and exiting rates.

Mortality of *Anopholes arabiensis* was consistently lower than that of *Anopholes gambiae* and *Anopholes funestus.* Mortality rates in trials with pyrethroid treated nets ranged from 25-52% for *Anopholes arabiensis*, 63-88% for *Anopholes gambiae* and 53-78% for *Anopholes funestus.*

LLINs and ITNs treated with pyrethroids were more effective at killing *Anopholes gambiae* and *Anopholes funestus* than *Anopholes arabiensis*. This could be a major contributing factor to the species shifts observed in East Africa following the scale up of LLINs.

With continued expansion of LLIN coverage in Africa *Anopholes arabiensis* is likely to remain responsible for residual malaria transmission, and species shifts might be reported over larger areas.

Experimental hut, Tanzania.

Combined use of Indoor Residual Spraying (IRS) and Long-Lasting Insecticidal Nets (LLINs) for malaria reduction in endemic rural Tanzania.

***LSHTM Investigators**: Natacha Protopopoff, Philippa West, Alexandra Wright, Immo Kleinschmidt & Mark Rowland.*
***External Investigators/Collaborators:** Frank Mosha & Reginald Kavishe (Kilimanjaro Christian Medical Colleges, Tanzania), Robert Malima & William Kisinza (National Institute for Medical Research, Tanzania).*

***Funding Body:** President's Malaria Initiative.*

Numerous studies have shown that Long Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) are effective in preventing malaria. What is less certain is the added value of combining both. The present study aims to evaluate the impact of combining IRS and LLINs compared to LLINs alone on malaria prevalence through a two-arm cluster randomized trial. The study is being carried out in Muleba district to the west of Lake Victoria in Tanzania. During this baseline year both arms of the study area received IRS with pyrethroid and universal LLIN coverage. IRS coverage reaches 95% of households and net usage increased from 41% to 56% after the LLIN mass distribution campaign. Result from two household and malaria prevalence cross-sectional surveys among children under 15 years showed an overall prevalence of 9.3% (5000 children) in February and 22.8% in July (4315 children). Seven monthly rounds of cross sectional mosquito collection using CDC light traps were conducted between April and December. A total of 12819 mosquitoes were collected of which 46% were *Anopheles*. A high frequency of resistance to pyrethroid and DDT was observed among *Anopheles gambiae* with mortality to these insecticides in WHO tests less than 40%. During the intervention year (2012), two more cross sectional malaria prevalence surveys are planned and 12 rounds of light trap collection.

Indoor Residual Spraying (IRS) and Long-lasting Insecticide Treated Nets (LLIN): Integration of methods and insecticide mode of actions for control of African malaria vector mosquitoes.

***LSHTM Investigators**: Fredros Okumu & Sarah Moore.*

***External Investigators/Collaborators:** John Grieco & Nicole Achee.*

***Funding Body:** USAID.*

Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) are the preferred techniques for malaria vector control in Africa, where their application has already contributed to significant reductions in the burden of the disease. Even though both methods are commonly used together in the same households, evidence of greater health benefits due to these combinations as opposed to use of either ITNs or IRS alone has been minimal and inconclusive.

The main aim of this research was therefore to contribute to this essential evidence, by way of experimental hut studies and mathematical simulations. We investigated whether there would be any added protective advantages when any of three selected Long Lasting Insecticidal Nets (LLINs) are combined with any of three selected IRS chemicals, as opposed to using any of the treatments alone. Data generated from the experimental hut studies was then inputed into an optimised deterministic mathematical model, simulating a typical malaria endemic village.

Both the field studies and the simulations showed that any synergies or redundancies resulting from LLIN/IRS combinations are primarily a function of modes of action of active ingredients used in the two interventions. Where LLINs are already present, addition of IRS would be redundant unless the IRS chemical is highly toxic, but where IRS is the pre-existing intervention, these combinations always confer improved protection. Therefore, IRS households should always be supplemented with nets, preferably LLINs, which not only protect house occupants against mosquito bites, but also kill additional mosquitoes. Finally, where resources are limited, priority should be given to providing everybody with LLINs and ensuring that these nets are consistently and appropriately used, rather than trying to implement both LLINs and IRS in the same community at the same time.

Combining Indoor Residual Spraying (IRS) with chlorfenapyr and Long-Lasting Insecticide Nets (LLINs) for improved control of pyrethroid-resistant Anopheles gambiae: an experimental hut trial in Benin.

***LSHTM Investigators**: Corine Ngufor, Raphael N'Guessan & Mark Rowland.*

***External Investigators/Collaborators:** Pelagie Boko, Abibatou Odjo, Estelle Vigninou, Alex Asidi & Martin Akogbeto (Centre de Recherches Entomologiques de Cotonou, Benin).*

***Funding Body:** The Bill & Melinda Gates Foundation through the Innovative Vector Control Consortium.*

Neither Indoor Residual Spraying (IRS) nor Long-Lasting Insecticidal Nets (LLINs) are able to fully interrupt transmission in holoendemic Africa as single interventions. The combining of IRS and LLINs presents an opportunity for improved control and management of pyrethroid resistance through the simultaneous presentation of unrelated insecticides. Chlorfenapyr IRS and a pyrethroid-impregnated polyester LLIN (WHO approved) were tested separately and together in experimental huts in southern Benin against pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus*. *Anopheles gambiae* were genotyped for the *kdr* gene to assess the combination's potential to prevent the selection of pyrethroid resistance.

The frequency of *kdr* was 84%. The overall mortality rates of *Anopholes gambiae* were 37% and 49% with the six-hole and 80-hole LLINs, respectively, and reached 57% with chlorfenapyr IRS. Overall mortality rates were significantly higher with the combination treatments (82-83%) than with the LLIN or IRS individual treatments. Blood feeding (biting) rates and repellency of mosquitoes with the combination of LLIN and chlorfenapyr IRS showed significant improvement compared to the IRS treatment but did not differ from the LLIN treatments indicating that the LLINs were the primary agents of personal protection. The combination killed significantly higher proportions of *Culex quinquefasciatus* (51%, 41%) than the LLIN (15%, 13%) or IRS (32%) treatments.

The chlorfenapyr IRS component was largely responsible for controlling pyrethroid-resistant mosquitoes and the LLIN component was largely responsible for blood feeding inhibition and personal protection. Together, the combination shows potential to provide additional levels of transmission control and personal protection against pyrethroid-resistant mosquitoes, thereby justifying the additional resources required. Chlorfenapyr has potential to manage pyrethroid resistance in the context of an expanding LLIN/IRS strategy.

Investigating novel indoor delivery systems for insecticides against malaria vectors.

***LSHTM Investigators**: Corine Ngufor, Raphael N'Guessan & Mark Rowland.**External Investigators/Collaborators:** Patrick Tungu (National Institute for Medical Research, Tanzania); Benjamin Koudou (Centre Suisse de Recherche Scientifique, Cote D'Ivoire); Sagnon N'Fale (Centre National de Recherche et de Formation sur le Paludisme, Burkina Faso).*

***Funding Body:** The European Union Seventh Research Framework Programme through the AvecNet Consortium.*

Despite the considerable increase in donor funding to support the global drive towards malaria elimination, it is unlikely that Indoor Residual Spraying (IRS) due to its associated operational and logistic constraints would be scaled-up to satisfactory levels to effectively interrupt transmission in holoendemic areas in sub-Saharan Africa. Alternative effective tools for delivering insecticides in doors which can be deployed either as single interventions or to complement Long Lasting Insecticidal Nets (LLINs). LLINs need to be urgently investigated and developed.

The aim of this study is to evaluate plastic wall linings and net wall hangings treated with the organophosphate pirimiphos methyl as novel malaria vector control tools. Insecticide treated plastic sheeting and net wall hangings can be likened to a long-lasting IRS treatment and also have the advantage of providing a more uniform covering of the wall with insecticide compared to IRS and of improving interior appearance of traditional dwellings. Their potential to provide improved vector control and/or manage insecticide resistance when deployed to complement LLINs in a combination approach will also be investigated.

Experimental hut trials are being carried out in 3 malaria endemic areas in Sub-Saharan Africa including Muheza-Tanzania where the vectors are largely susceptible to insecticides, Valley du Kou-Burkina Faso where the vectors are very resistant to pyrethroids but susceptible to organophospates and Tiassales-Cote D'Ivoire where pyrethroid and organophosphate resistance co-exist.

Evaluation of the long-lasting organophosphate pirimifos methyl CS for Indoor Residual Spraying (IRS) against pyrethroid-resistant Anopheles gambiae Giles and Culex quinquefasciatus: an experimental hut trial in Southern Benin.

***LSHTM Investigators**: Raphael N'Guessan & Mark Rowland.*
***External Investigators/Collaborators:** Pelagie Boko, Abibatou Odjo, Estelle Vignonou, Hermione Adje, Alex Asidi & Martin Akogbeto (Centre de Recherche Entomologique de Cotonou, Benin).*

***Funding Body:** The Bill & Melinda Gates Foundation through the Innovative Vector Control Consortium.*

There is an urgent need to develop safe, long- lasting alternatives to DDT and pyrethroids to sustain malaria vector control and reduce selection pressure for pyrethroid resistance.

Two formulations of pirimiphos methyl (CS) applied as an IRS treatment were evaluated in experimental huts in an area of southern Benin where *Anopheles gambiae* and *Culex quinquefasiactus* are resistant to pyrethroids but susceptible to organophosphates. Dosages tested were 1g/m2 and 0.5g/m2 against standard pirimiphos methyl EC for up to 12 months on mud and cement walled substrates.

In mud huts 1 g/m2 was clearly superior to 0.5 g/m2 and induced >50% mortality of *Anopheles gambiae* for more than 10 months. In cement walled huts 0.5 g/m2 induced high levels of control for almost one year. Overall, the BM formulation seemed superior to B formulation. The EC formulation of pirimiphos methyl sprayed at 1 g/m2 was giving inadequate control of *Anopheles gambiae* within 2 months of spraying. A microencapsulated formulation of lambdacyhalothrin (Icon CS) was sprayed as a positive control and although it showed prolonged residual activity in cone tests on walls using a susceptible reference strain, it was unable to control freely entering pyrethroid resistant *Anopheles gambiae*. Pirimiphos methyl CS was also found to be highly effective against C*ulex quinquefasciatus*, giving between 90-50% control for up to 10 months at 1 g/m2 on cement surfaces and for 6 months on mud surfaces.

Pirimiphos methyl CS showed great promise for control of pyrethroid-resistant *Anopheles gambiae* in West Africa and for delaying development of pyrethroid resistance where LLINs are already widely used. A cost effective alternative to DDT, giving year-round transmission control in endemic rural settings in Africa is now a realistic prospect.

Control of pyrethroid and DDT-resistant Anopheles gambiae by application of Indoor Residual Spraying (IRS) or mosquito nets treated with a long-lasting organophosphate insecticide, chlorpyrifos-methyl.

***LSHTM Investigators**: Raphael N'Guessan & Mark Rowland.*

***External Investigators/Collaborators:** P Boko, A Odjo, J Chabi, M Akogbeto (Centre de Recherche Entomologique de Cotonou, Benin).*

***Funding Body:** The Bill & Melinda Gates Foundation through the Innovative Vector Control Consortium.*

Dow Agrosciences have developed a microencapsulated formulation (CS) of the organophosphate chlorpyrifos methyl as a cost-effective, long-lasting alternative to DDT.

We tested this product as an IRS or ITN treatment in experimental huts in an area of Benin where *Anopheles gambiae* and *Culex quinquefasiactus* are resistant to pyrethroids, but susceptible to organophosphates. Efficacy and residual activity was compared to that of DDT and the pyrethroid lambdacyalothrin.

IRS with chlorpyrifos methyl killed 95% of *Anopheles gambiae* that entered the hut as compared to 31% with lambdacyhalothrin and 50% with DDT. Control of *Culex quinquefasciatus* showed a similar trend; although the level of mortality with chlorpyrifos methyl was lower (66%) it was still much higher than for DDT (14%) or pyrethroid (15%) treatments. Nets impregnated with lambdacyhalothrin were compromised by resistance, killing only 30% of *Anopheles gambiae* and 8% of *Culex quinquefasciatus*. Nets impregnated with chlorpyrifos methyl killed more (45% of *Anopheles gambiae* and 15% of *Culex quinquefasciatus*, but its activity on netting was of short duration. Contact bioassays on the sprayed cement-sand walls over the nine months of monitoring showed no loss of activity of chlorpyrifos methyl, whereas lambdacyhalothrin and DDT lost activity within a few months of spraying.

The remarkable residual activity indicates that cost-effective alternatives to DDT are feasible through modern formulation technology.

Mosquito net, Tanzania

Mount Kilimanjaro, Tanzania.

An experimental hut evaluation of PermaNet® 3.0, a deltamethrin–piperonyl butoxide combination net, against pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus mosquitoes in southern Benin.

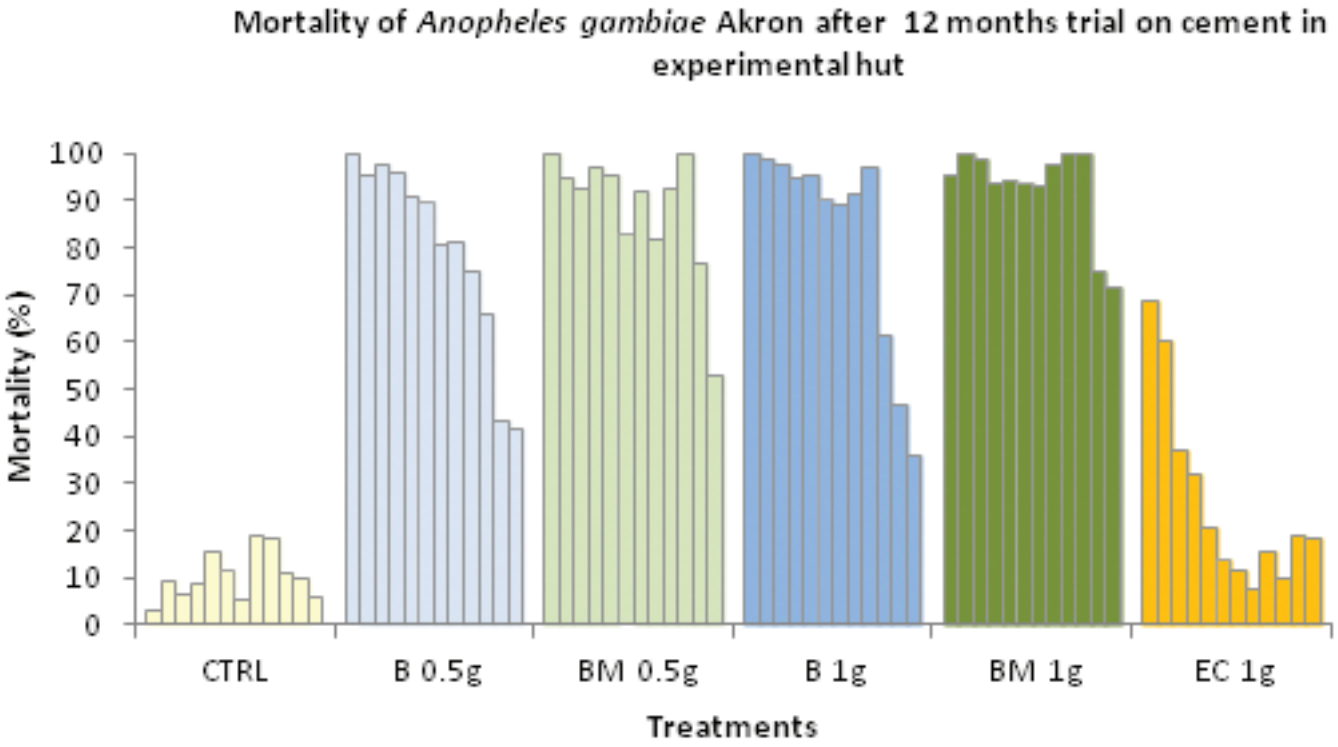
***LSHTM Investigators**: Raphael N'Guessan, Alex Asidi & Mark Rowland.*

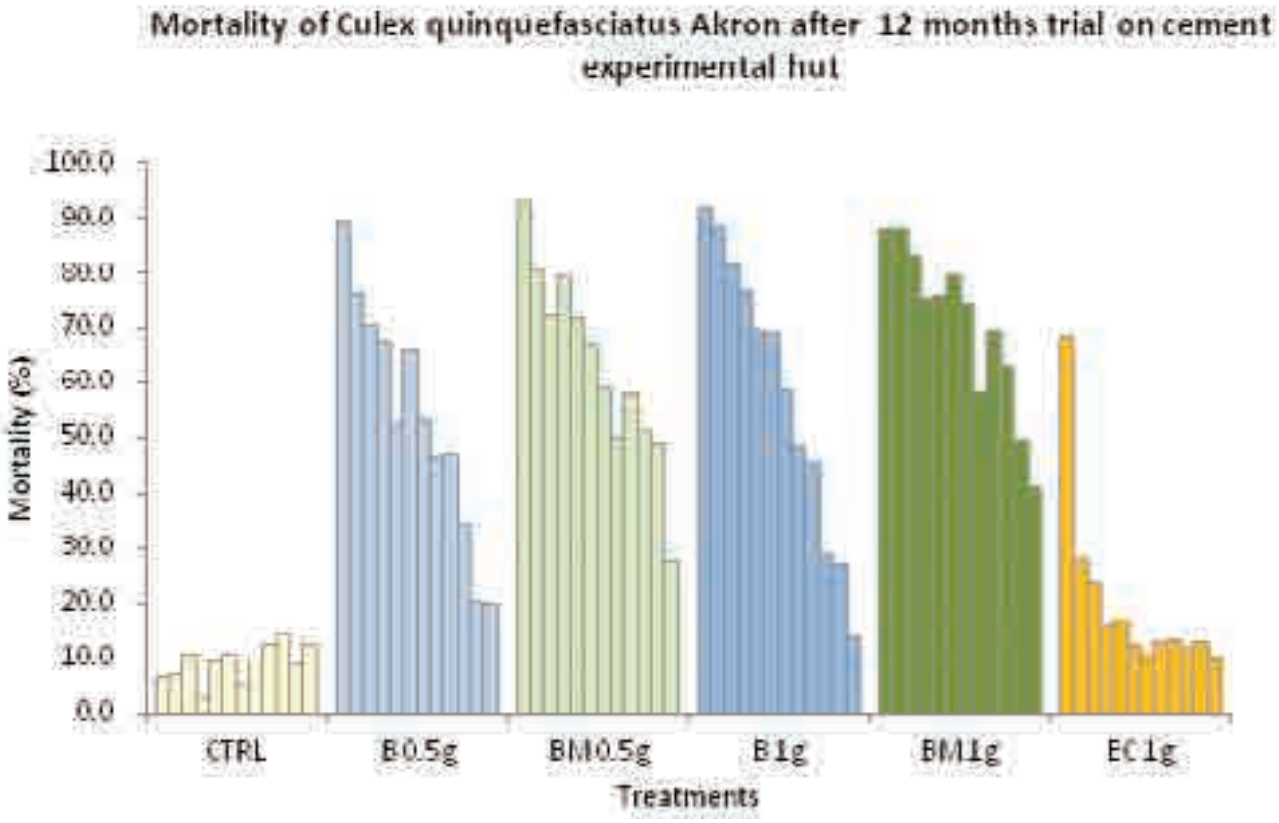
***External Investigators/Collaborators:** P Boko, A Odjo, M Akogbeto, O Pigeon.*

***Funding Body:** Vestergaard Frandsen.*

PermaNet 3.0 is a long-lasting combination net with deltamethrin present on the sides and a mixture of deltamethrin and piperonyl butoxide (PBO), an oxidase synergist, on the top panel.

An experimental hut trial comparing unwashed and 20 times washed PermaNet 3.0 and PermaNet 2.0, Olyset Net and a conventional deltamethrin-treated net washed three times was conducted in southern Benin. *Anopheles gambiae* and *Culex quinquefasciatus* from this area are highly resistant to pyrethroids through *kdr* and cytochrome P450 mechanisms. The unwashed PermaNet 3.0 killed slightly more A. gambiae (52%) than the unwashed PermaNet 2.0 (44%) (P=0.036), indicating only partial synergism of resistance. After washing there was significant loss of activity to a similar level, with PermaNet 3.0 killing 31%, PermaNet 2.0 killing 29% and the conventional net killing 26%. Blood-feeding rates were partially inhibited for unwashed PermaNet 3.0 and Olyset Net (27% inhibition). Personal protection against *Anopholes gambiae* derived from PermaNet 3.0 was similar to that from PermaNet 2.0 before washing (50% vs. 47%), and after 20 washes it decreased to 30%. Against *Culex quinquefasciatus,* no treatment killed >24% entering the huts. The synergism from unwashed PermaNet 3.0 was lower than expected, probably due to an unidentified resistance mechanism unaffected by PBO.


Mortality of *Anopheles gambiae* Akron after 12 months trial on cement in experimental hut
Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
CTRL
B 0.5g
BM 0.5g
B 1g
BM 1g
EC 1g
Treatments


Mortality of Culex quinquefasciatus Akron after 12 months trial on cement experimental hut
Mortality (%)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
CTRL
B0.5g
BM0.5g
B1g
BM1g
EC1g
Treatments

Study site, Tanzania.

Health promotion for impoverished rural and refugee populations in Tanzania focusing on malaria control, sanitation and water supply.

***LSHTM Investigators**: Frank Mng'ong'o, Jeroen Ensink & Sarah Moore.*

***External Investigators/Collaborators:** Joseph Sambali (Ifakara Health Institute, Tanzania); Jaka Magonga (Concern Worldwide, Tanzania); Ann Le Mare (University of Durham, UK).*

***Funding Body:** Concern Worldwide Tanzania.*

In line with Concern's goal to "help people living in extreme poverty achieve major improvements in their lives which last and spread without ongoing support from Concern", we aim to develop and institutionalize research support for Concern's activities at the Ifakara Health Institute through an active operational research programme focusing on preventing malaria and water-borne diseases.

Sustained malaria control is underway using a combination of vector control, prompt diagnosis and treatment of malaria cases. Progress is excellent, but for long-term control, low-cost, sustainable tools that supplement existing control programs are needed. Conventional vector control tools such as Indoor Residual Spraying and house screening are highly effective, but difficult to deliver in rural areas. Therefore, an additional means of reducing mosquito house entry was evaluated: the screening of mosquito house entry points by planting the tall and densely foliated repellent plant Lantana camara L. around houses.

A pilot efficacy study was performed in Kagera Region, Tanzania in an area of high seasonal malaria transmission, where consenting families within the study village planted L. camara (Lantana) around their homes and were responsible for maintaining the plants. Questionnaire data on house design, socioeconomic status, malaria prevention knowledge, attitude and practices was collected from 231 houses with Lantana planted around them 90 houses without repellent plants. Mosquitoes were collected using CDC Light Traps between September 2008 and July 2009. Data were analysed with generalised negative binomial regression, controlling for the effect of sampling period.

Indoor catches of mosquitoes in houses with Lantana were compared using the Incidence Rate Ratio (IRR) relative to houses without plants in an adjusted analysis. There were 56% fewer *Anopheles gambiae* s (IRR 0.44, 95% CI 0.28–0.68, p,0.0001); 83% fewer *Anopheles funestus* (IRR 0.17, 95% CI 0.09–0.32, p,0.0001), and 50% fewer mosquitoes of any kind (IRR 0.50, 95% CI 0.38–0.67, p,0.0001) in houses with Lantana relative to controls.

House screening using Lantana reduced indoor densities of malaria vectors and nuisance mosquitoes with broad community acceptance. Providing sufficient plants for one home costs USD 1.50 including maintenance and labour costs, (30 cents per person).

Field efficacy of pyrethroid treated plastic sheeting (durable lining) in combination with Long Lasting Insecticidal Nets (LLINs) against malaria vectors.

***LSHTM Investigators**: Raphael N'Guessan, Seth Irish & Mark Rowland.*
***External Investigators/Collaborators:** F Chandre, J.M Hougard & L Djogbenou (Institut de Recher pour le development); R.K Dabire (Centre Muraz, Burkina faso).*

***Funding Body:** Sumitomo Chemical.*

Insecticide Treated Plastic Sheeting (ITPS), sometimes known as durable lining, has potential as a long-lasting insecticidal surface for malaria vector control indoor. We examined the effect of combining ITPS with LNs in experimental huts in Burkina Faso. Two molecular forms of *Anopheles gambiae* (S and M) coexist, with *kdr* resistance more prevalent in S. The treatment arms included ITPS, Olyset, ITPS plus Olyset, ITPS plus untreated net (with or without holes), and untreated control.

ITPS was significantly inferior to Olyset LN in terms of mortality (37% vs 63%), blood feeding inhibition (20% vs 81%) and deterrence (0 vs 42%) effects, and hence altogether inferior as a means of personal protection (16% vs 89%). The addition of ITPS to Olyset did not improve mortality (62%), blood feeding inhibition (75%), deterrence (50%) or personal protection (88%) over that of Olyset used alone. Use of untreated nets - both holed and intact - with ITPS provided greater protection from blood-feeding. The intact net/ITPS combination killed more mosquitoes than ITPS on its own.

The combination of pyrethroid IRS and pyrethroid LN - as practiced in some countries - is unlikely to be additive or synergistic. A combination of LN and ITPS treated with an alternative insecticide is likely to be more effective, particularly in areas of pyrethroid resistance.

Multiple Phase III Trials.

***LSHTM Investigators**: Matt Kirby, Mark Rowland & Patrick Tungu.*
***External Investigators/Collaborators:** William Kisinza, Robert Malima & Stephen Magesa (National Institute for Medical Research, Tanzania).*

***Funding Body:** Sumitomo Corporation (PRISM) & WHO Pesticide Evaluation Scheme (all other trials).*

The PRISM, Icon®Maxx and InterceptorTM trials are ongoing household or cluster randomized controlled trials of insecticide treated materials (bed nets and bed sheets) in rural hamlets and villages close to Muheza. There are considerable similarities in the protocols of these trials; namely, the products are being evaluated in terms of entomological efficacy, durability and acceptability, during use in the communities by participants blinded to the type of net/sheet received, for a period between 6 months and 3 years. Sheets and nets are retrieved from the field periodically and tested in cone and tunnel bioassays against susceptible Anopheles gambiae Kisumu. The frequency of washing is also determined and samples are subjected to HPLC to determine AI residue.

Pyrethroid and Repellent Insecticidal Sheets against Malaria (PRISM).

Bed sheets treated with Scoron® (Cyphenothrin) were given to 1205 households (universal coverage) whilst another 1246 households received otherwise identical untreated sheets prior to the main transmission season of 2011. Pre- and post-intervention surveys recorded malaria and anaemia prevalence from 2450 children aged 6 months - 13 years, whilst light trap catches from 240 sentinel houses measured vector densities in both arms of the trial. Focus group discussions, bioassays and chemical analyses of sample surveys determined durability and acceptability. Though some bioassays and analyses are still ongoing, it is clear that there are problems with the product. Laboratory tests show that new sheets are both repellent and insecticidal, but that these effects are lost over several rounds of washing. In the field no difference in *Anopheles gambiae* density was observed between intervention and control clusters though relative to the dry season the increase in total mosquitoes in the rainy season was less marked in the intervention clusters compared to the control. Fewer children in Scoron® clusters were anaemic and the haemoglobin density was higher compared to the control clusters, though differences were slight 4.6% and 11.03g/dL v 5.5% and 10.97g/dL) & there was no significant difference in parasite prevalence (24% v 28%, p >0.05). On average sheets in the community were washed once every two weeks and it is thought that inadequacy of the insecticide binder to prevent loss of insecticide during washing, plus inconsistent/incorrect use of the sheets, limit the performance of the product.

Insecticidal efficacy and household acceptability of Icon® Maxx long-lasting treatment for nets.

This re-treatment kit consists of a slow-release lambda-cyhalothrin capsule suspension. Nets treated with Icon®Maxx and conventional nets with the same insecticide (IconNet) have been distributed randomly to 818 households within two villages, for which baseline data was collected in 2011. Participants were blinded to which type of net they received.

Net pieces from Icon Maxx and Icon Net treated but not distributed (i.e. baseline AI) have been prepared for HPLC analysis and tested in cone bioassays. The IconMaxx nets at baseline have 99.9% killing efficacy and 100% knockdown efficacy. The trial is ongoing: nets of both types are now being retrieved from the field every 6 months.

Insecticidal efficacy and household acceptability of InterceptorTM long-lasting treated nets.

This long lasting insecticidal net contains a finishing product that binds the alphacypermethrin insecticide in a special coating to the fibres of the nets. Interceptor LN received a WHO interim recommendation for use in malaria control in 2007. It then entered a 3 year community trial carried out by LSHTM and NIMR during 2009-2011 to determine whether the product can receive a full recommendation. Interceptor nets and conventional insecticide treated nets (the control) were distributed at random to households in rural 3 villages in Muheza Tanzania. Every 6 months nets were sampled for bioassay and assessment of net durability and insecticide content ,and householders were surveyed for washing practices, net usage and adverse effects. Nets years that failed the cone bioassay criteria were assessed in tunnel bioassays. Nets were regularly used and washed, and no adverse effects were reported after the first month of use. After 3 years only 13% of the Interceptor TM nets were failing the bioassay criteria whereas after just one year of use 37% of the conventional treated net were failing. After review by the WHO expert committee InterceptorTM was granted full recommendation as a long lasting insecticidal net.

Mosquito nets and sheets.

Multiple Phase II Trials.

***LSHTM Investigators**: Matt Kirby, Mark Rowland & Patrick Tungu.*
***External Investigators/Collaborators:** William Kisinza, Robert Malima & Stephen Magesa (National Institute for Medical Research, Tanzania).*

***Funding Body:** Sumitomo Corporation (PRISM) & WHO Pesticide Evaluation Scheme (all other trials).*

All trials follow the WHO guidelines for laboratory and field testing of LNs (WHO/CDS/WHOPES/GCDPP/2005.11). Six dentical experimental huts specially designed for recording the entering and exiting behaviour of mosquitoes and for measuring responses to insecticide-treated materials (nets and sheets) were used for all trials. The standard procedure in these trials was to test the insecticide-treated material under evaluation, unwashed and washed 20x, against conventional treated material (+ve control) and untreated material (-ve control). These materials were rotated weekly between huts. The outcome measures were deterrence, repellence, immediate and delayed mortality, and induced bloodfeeding inhibition. Pieces are cut from all materials before and after the trial to measure the concentration of active ingredients.

DawaPlus 2.0 LN.

Long-lasting deltamethrin-coated polyester bed net. DawaPlus outperformed conventional insecticide-treated nets in laboratory assays of mosquito mortality, but these differences were less marked in experimental huts. However, Dawa Plus nets (unwashed and 20x washed) did give better personal protection than 3x washed conventional nets. Bloodfeeding inhibition was 63% for DawaPlus nets and there was no difference between washes implying that the deltamethrin coating had a high degree of wash resistance.

Efficacy of Cyphenothrin (Scoron®) and DEET-MC treated bed sheets.

Bed sheets treated with Cyphenothrin (250mg/m2) or 1 of 2 concentrations (4 & 8g/m2) of micro-encapsulated DEET. For *Anopheles* mosquitoes, deterrence (11-33% increase), mortality (33-47% increase) and repellency were higher for all treated materials compared to the untreated control. The 8g/m2 DEET sheet induced significantly higher mortality than all other treated sheets. Feeding inhibition was moderate to high (25-88%) for all treated materials against Anopheles and Culex mosquitoes

Evaluation of Olyset Plus LN against Anopheles gambiae arabiensis and Culex quinquefasciatus in experimental huts.

Permethrin & Oxidase synergist Piperonyl butoxide. Trial is assessing whether bed nets treated with this combination show enhanced activity against insecticide-susceptible *Anopheles* and resistant *Culex quinquefasciatus*. Trial is ongoing.

Replacing DDT: Rigorous evaluation of spatial repellents for the control of vector borne diseases.

***LSHTM Investigators**: Sheila Ogoma, Peter Sangoro, Marta Maia, Lena Lorenz, Ann Kelly, Raphael N'Guessan & Sarah Moore.*

***Funding Body:** The Bill & Melinda Gates Foundation.*

Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) are the preferred techniques for malaria vector control in Africa, where their application has already contributed to significant reductions in the burden of the disease. However, there remains a proportion of malaria transmission that is transmitted by early evening feeding mosquitoes that bite and rest outdoors. One solution to this problem is spatial repellents that continuously protect people within a space from mosquito bites without the need for people to remember to apply repellents.

The main aim of this research is to devise effective means of quantifying the effects of spatial repellents, as they are a new paradigm in the field of vector control. The research will provide industry with a target product profile (TPP) for spatial repellents to advise on the development of new and effective vector control products.

Both field and laboratory assays have been developed to characterise the mode of action of spatial repellents. This will be used to inform new WHO spatial repellent guidelines that are currently being developed. Data on the TPP will be available later in the year.

Update on resistance status of Anopheles gambiae to conventional insecticides at a previous WHOPES field site, "Yaokoffikro", 6 years after the political crisis in Côte. d'Ivoire.

***LSHTM Investigators**: Raphael N'Guessan.*

***External Investigators/Collaborators:** Alphonsine Koffi, Ludovic Alou, Maurice Adja, Moussa Koné (Institut Pierre Richet, Bouake, Côte d'Ivoire) ; Fabrice Chandre (Institut de Recherche pour le Development, France).*

***Funding Body:** Ministere Cooperation Française.*

At Yaokoffikro field site near Bouaké, in central Côte d'Ivoire, group of experimental huts built in 1996 served over many years for the evaluation of insecticides against highly resistant mosquitoes. Breeding sites of mosquitoes and selection pressure in the area were maintained by local farming practices until a war broke out in September 2002. Six years after the crisis, we conducted bioassays and biochemical analysis to update the resistance status of Anopheles gambiae s.s. population and detect other potential mechanisms of resistance that might have evolved.

High pyrethroids, DDT and carbamate resistance was confirmed in An. gambiae s.s. population from Yaokoffikro. Mortality rates were less than 70% with pyrethroids and etofenprox, 12% with DDT, and less than 22% with the carbamates. Tolerance to fenitrothion was observed, with 95% mortality after 24h.

PCR analysis of samples from the site showed high allelic frequency of the L1014F kdr (0.94) and the ace-1R (0.50) as before the crisis. In addition, increased activity of NSE, GST and to a lesser extent MFO was found relative to the reference strain Kisumu. This was the first report detecting enhanced activity of these enzymes in An. gambiae s.s from Yaokoffikro, which could have serious implication in detoxification of insecticides. Their specific roles in resistance should be investigated using additional tools.

The insecticide resistance profile at Yaokoffikro appears multifactorial. The site presents a unique opportunity to evaluate its impact on the protective efficacy of insecticidal products as well as new tools to manage these complex mechanisms. It calls for innovative research on the behaviour of the local vector, its biology and genetics that drive resistance.